CIRCADIAN RHYTHMICITY AND OTHER PATTERNS OF SPONTANEOUS MOTOR ACTIVITY IN *FRONTINELLA PYRAMITELA* (LINYPHIIDAE) AND *ARGYRODES TRIGONUM* (THERIDIIDAE)

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ABSTRACT. Endogenous biological rhythms are apparently found in all eukaryotic organisms. The most ubiquitous of these, the circadian rhythm, functions to synchronize physiology and behavior with diel changes in the environment. Data presented here demonstrate that, in the linyphiid spider *Frontinella pyramitela* (Walckenaer) and in the theridiid spider *Argyrodes trigonum* (Hentz), a circadian rhythm modulates locomotor activity in some individuals but not in others. The data also show (a) that higher-frequency endogenous rhythms play a part in determining the patterns of motor activity, and (b) that intervals between bouts of activity are influenced by aperiodic processes that appear to be stochastic.

Much of the behavior of animals in the field is closely correlated with environmental stimuli: the detection of predators or prey stimulates flight or pursuit, the presence of a rival results in agonism, the arrival of a mate elicits courtship or bonding rituals, and changes in the thermal environment lead to altered postures or positions. The internal state of an animal (e.g., the time since its last meal, the size of its gonads, or the presents of parasites and pathogens) also influences behavior, sometimes profoundly.

Among the most ubiquitous of internal state variables is the endogenous circadian clock, which has been particularly well studied since the pioneering work of Aschoff (1954) and Bünning (1963). Although the cellular and molecular bases of biological clocks remain obscure, their utility is well documented: they facilitate prediction of periodic environmental events, make celestial navigation possible, and enable the temporal organization of internal events. Because the overt manifestations of these clocks are often subtle, they are most easily and profitably studied in animals isolated from confounding environmental stimuli, including conspecifics and such *Zeitgebers* as light cycles. Under those conditions of isolation, an animal's behavior is said to be freerunning (i.e., showing the natural period of the circadian clock).

In spiders, endogenous rhythmicity has been suggested by data on diel rhythms under natural conditions (e.g., in *Amaurobius*, Cloudsley-Thompson 1957), and has been confirmed in a very few species under constant conditions (e.g., in *Cupiennius salei*, Seyfarth 1980; for other references, see Cloudsley-Thompson 1987). Moreover, in work with the linyphiid spider, *Frontinella pyramitela* (Walckenaer), my laboratory has found that internal programs quite distinct from circadian clocks appear to govern the timing of certain spontaneous (i.e., internally driven) behaviors related to courtship (Suter 1990) and cohabitation (Suter & Walberer 1989). The present study constitutes the beginning of an elucidation of the programs, both circadian and other, that underlie the onset and cessation of spontaneous behaviors in *F. pyramitela*.

METHODS

I captured adult females of both *F. pyramitela* and *A. trigonum* in Dutchess County, New York, during June, 1992, and immediately installed each in an open-ended glass cylinder. I placed the cylinder in an aluminum trough and inserted that into a plexiglass holder which could house eight such troughs. One end of each cylinder was partially occluded by an infrared sensitive phototransistor (Radio Shack SDP8403-301) and the other end was sealed by a circular microscope coverslip behind which was mounted an IR light-emitting diode (Radio Shack SEP8703). The assembled apparatus, with the simple electronic circuits used to drive it, is shown in Fig. 1. I enclosed the apparatus in a light-tight chamber (20 cm × 24 cm × 18 cm) in which the relative humidity was maintained at 100% and temper-
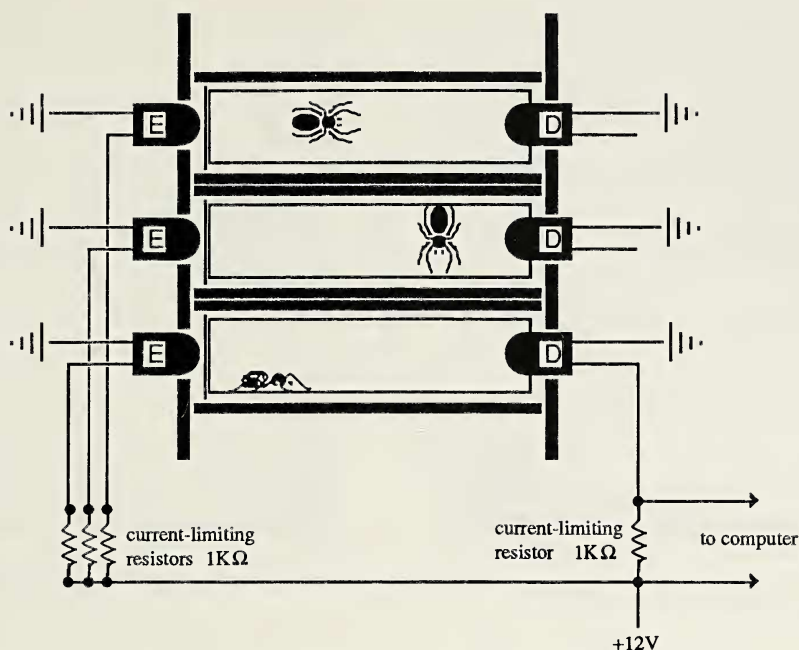
Figure 1.—Schematic diagram of the apparatus used to house spiders and detect their motion. IR emitters (E) and detectors (D), coupled to a computer, formed the motion-detection system. The spiders themselves were enclosed in glass cylinders 1 cm in diameter and 4.5 cm long.

ature was constant at 24 ±1 °C. The electronic driving circuits, which generate some heat, were mounted outside the chamber. Output from each IR phototransistor was digitized and recorded by computer (hardware: Macintosh IIci with National Instruments NB-MIO-16 I/O board; software: a customized data-logging program written in LabVIEW 2). At each of the eight channels, amplitude was measured at 2 ms intervals (500 Hz) and the standard deviation of the amplitudes collected during 1.6 s was the datum recorded as an index of activity during that period. (Preliminary tests had shown that, because of the multiple paths the IR light could take in a chamber, grooming motions and other movements of appendages did not cause fluctuations in phototransistor output. Thus high-frequency fluctuations were unlikely. Moreover, power spectra of phototransistor outputs from chambers containing rapidly moving spiders recorded at 500 Hz with no averaging revealed that there was no significant energy at frequencies > 1.0 Hz).

The activity of 14 *F. pyramitela* and 5 *A. trigonum* was recorded during the study. The maximum continuous length of time in the chambers was 11 days for a group of 7 *F. pyramitela*. While in the chambers, the spiders were neither fed nor watered. Because of the high relative humidity, desiccation was not a problem for the spiders, and fasting for periods longer than 11 days can be tolerated by *F. pyramitela* (Suter 1985) and by other species (Anderson 1974). Two separate experiments involving *F. pyramitela* were run: in one the spiders were maintained in constant darkness (DD) throughout their 8-day isolation, and in the other the chamber enclosing the spiders was lit for 8 hours each day for 11 days (LD, onset of light daily at 1100 h; illumination by orange, green, and red LEDs provided 7.7 lux at each chamber). The single experiment that involved *A. trigonum* was carried out in DD.

The digitized activity records of individual spiders were analyzed in two ways. (1) Spectral Analysis I: A Hamming window (to minimize the artifacts caused by truncation of a signal) was applied to the recorded string of activity/inactivity periods which was then analyzed by fast Fourier transform (FFT) and displayed as a power spectrum showing the proportion of explained variance in activity as a function of frequency. Significant peaks were identified by a chi-square method described elsewhere (Suter & Forrest, in press) which could reveal time-based periodicities such as a circadian rhythm. For the detection
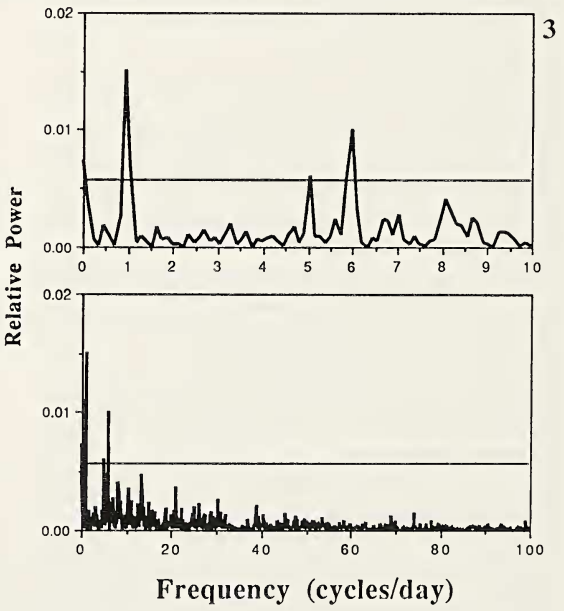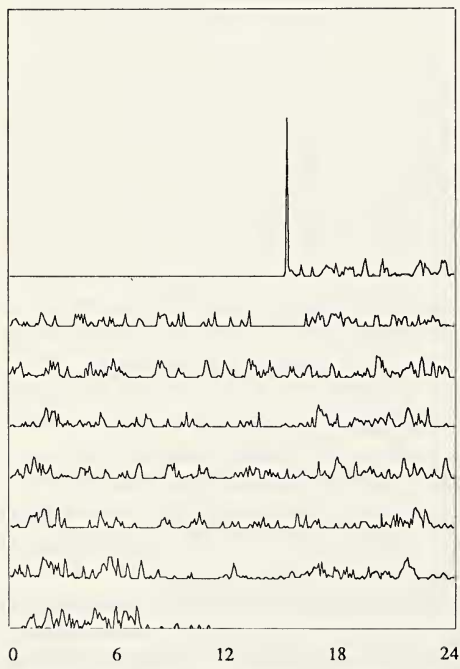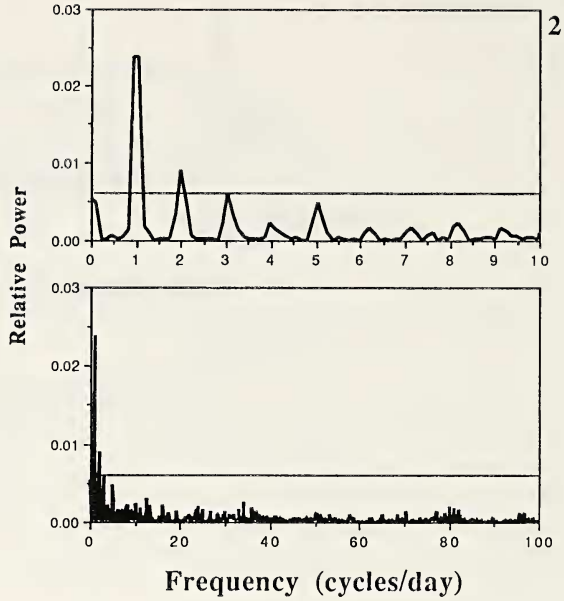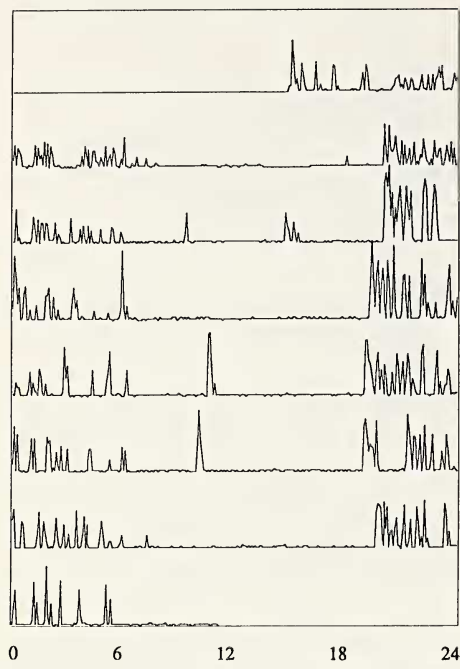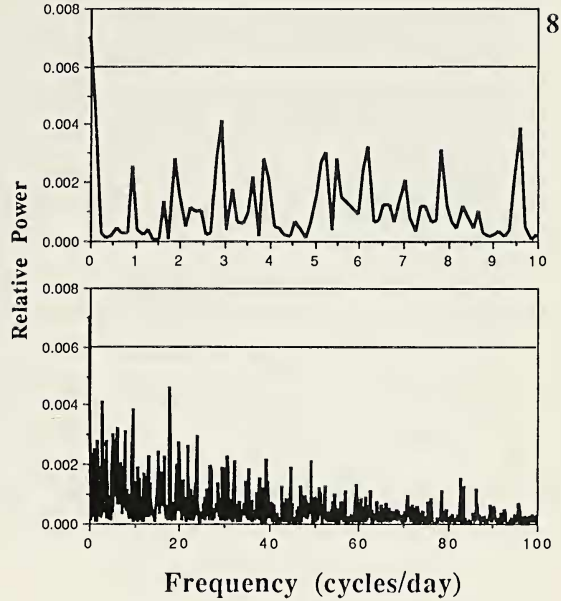
Figures 2–8.—Activity of *F. pyramitela* under conditions of constant darkness (DD). Graphs on the left show activity as a function of time, with days shown sequentially from top to bottom. FFT analysis of these data resulted in the power spectra shown on the right in each figure. The power spectra are shown at two scales to elucidate both the overall pattern (lower panel) and the peaks between 0 and 10 cycles/d (upper panel). In each of these power spectra (as in all others in this study), the first component (furthest to the left) should be ignored because it reflects the entire data set and not oscillations within the data set. Much of the explained variance is concentrated near 1.0 cycle/d, but significant peaks are also found at higher frequencies. Peaks at or above the horizontal lines are significant at $\alpha = 0.01$.
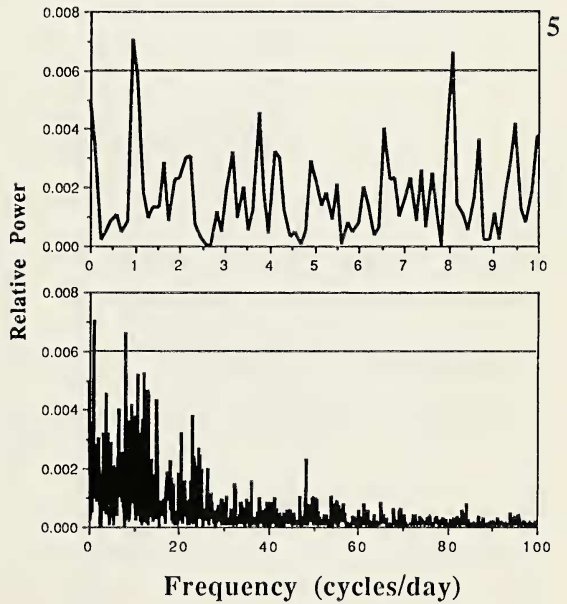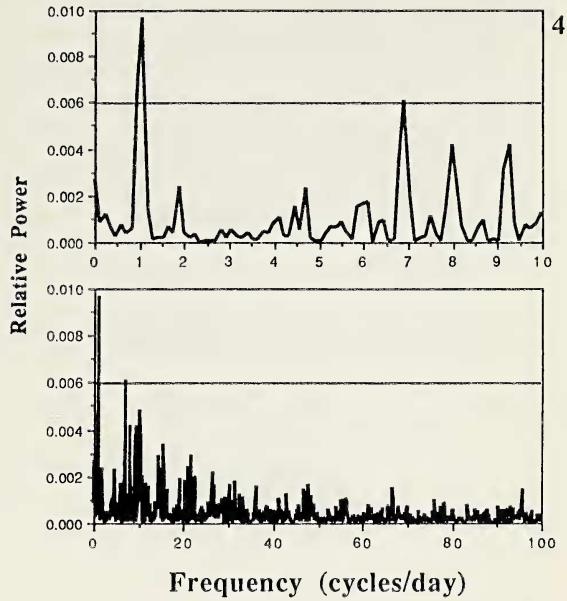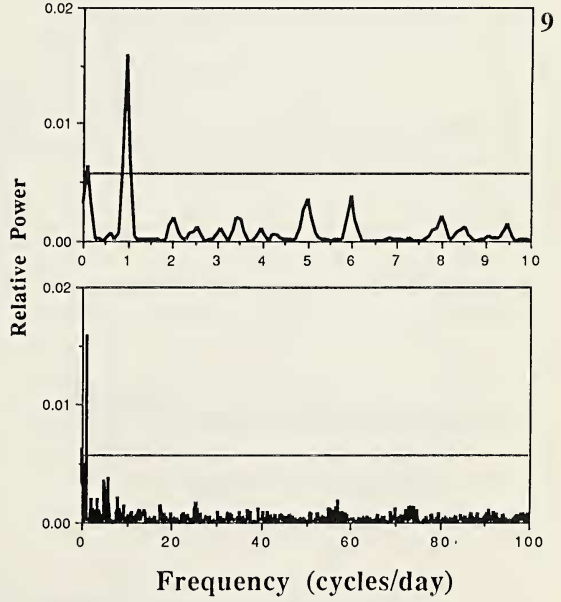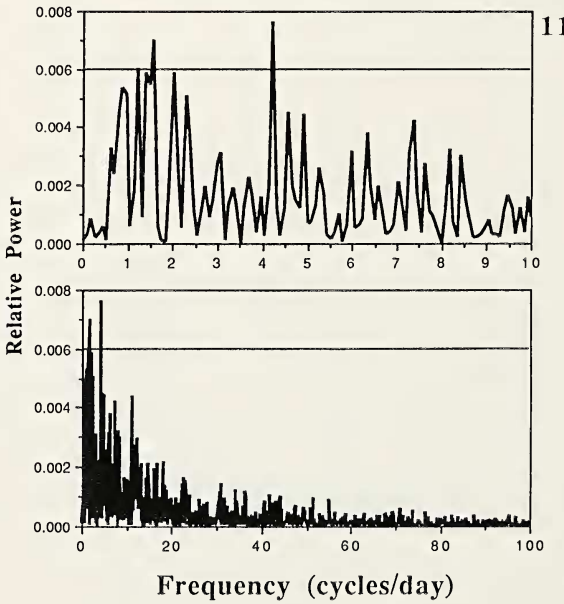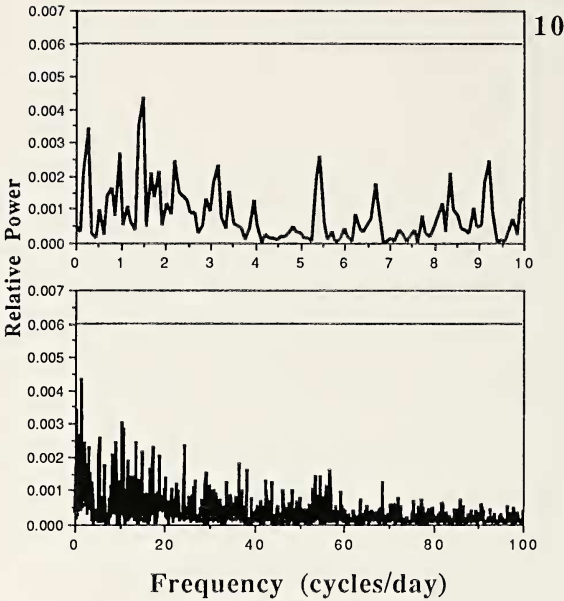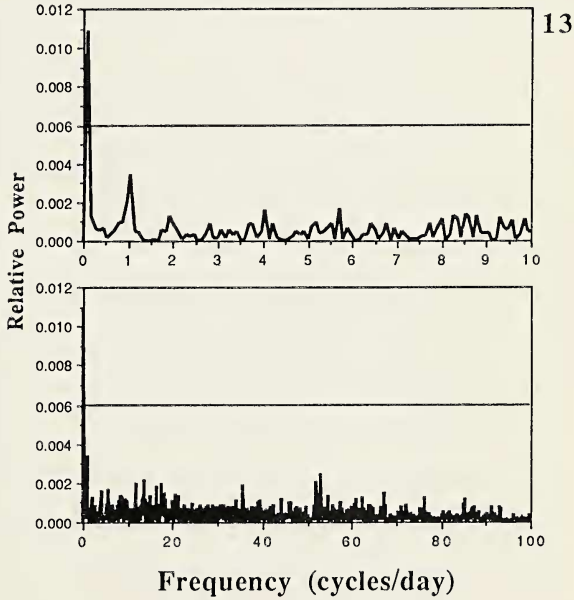
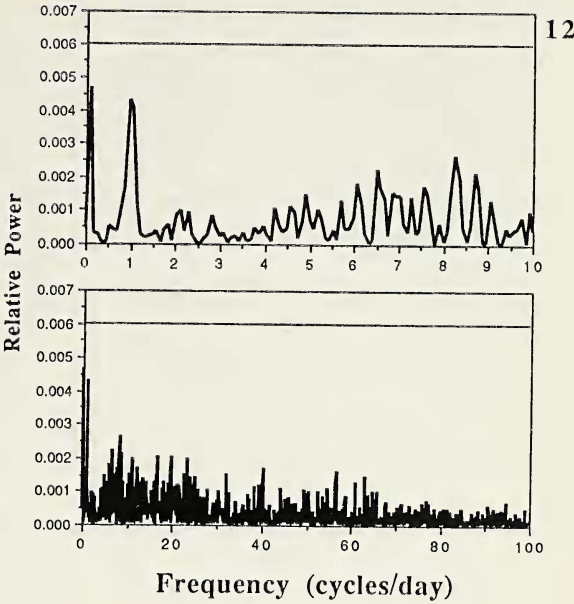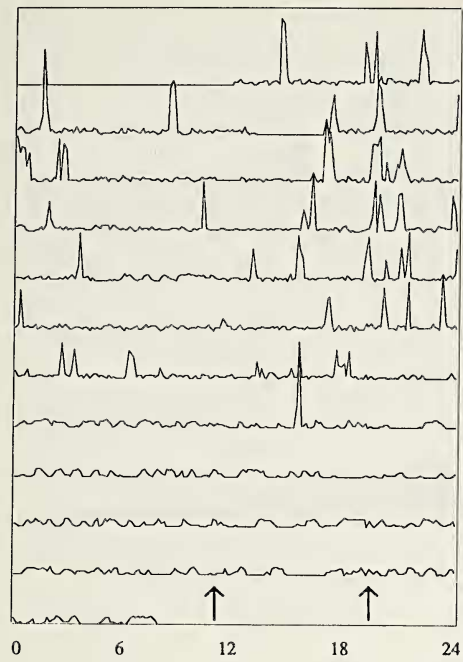Figures 2--8.—Continued.
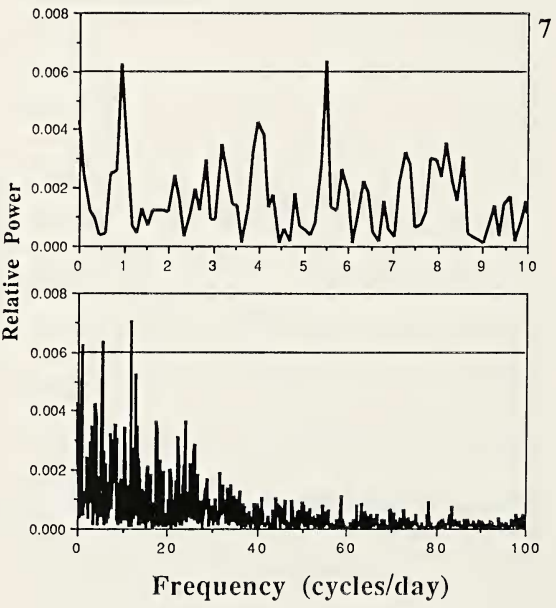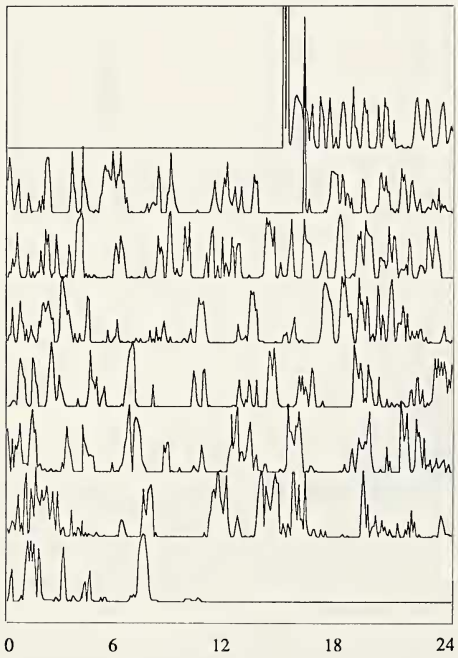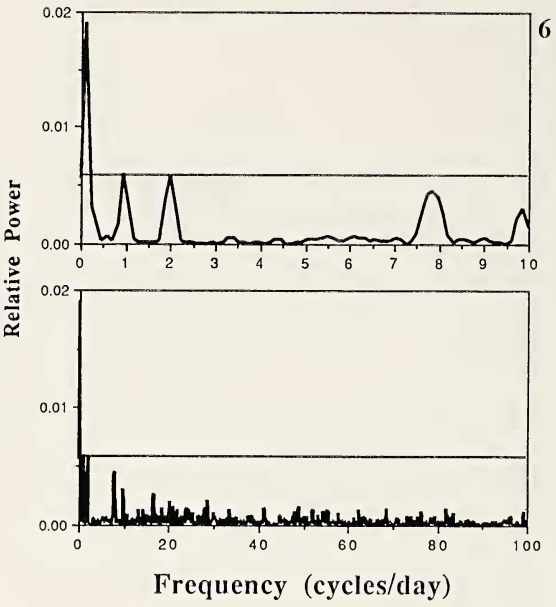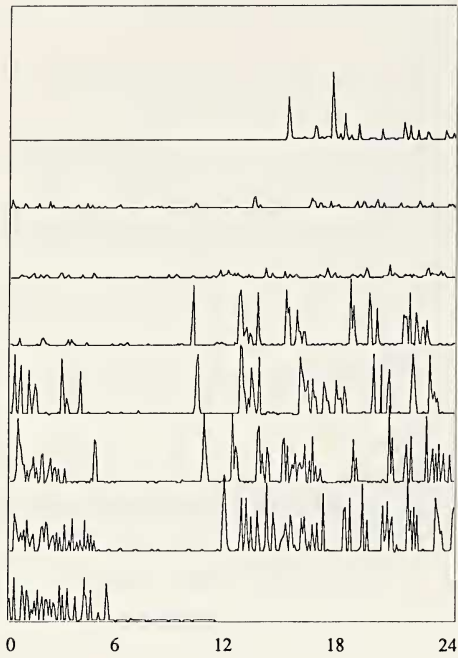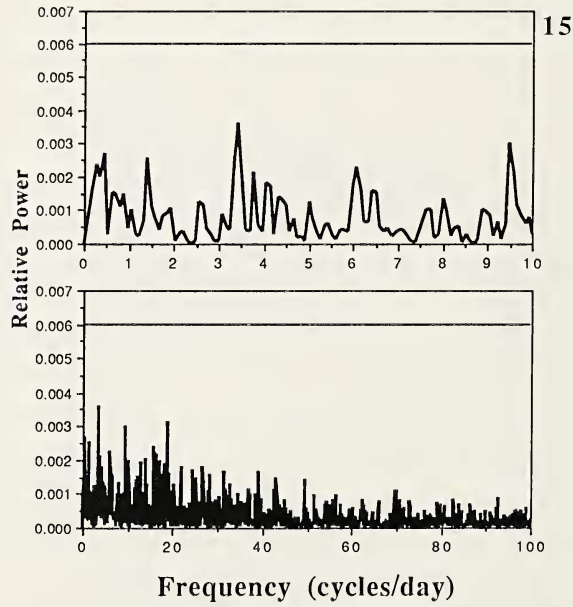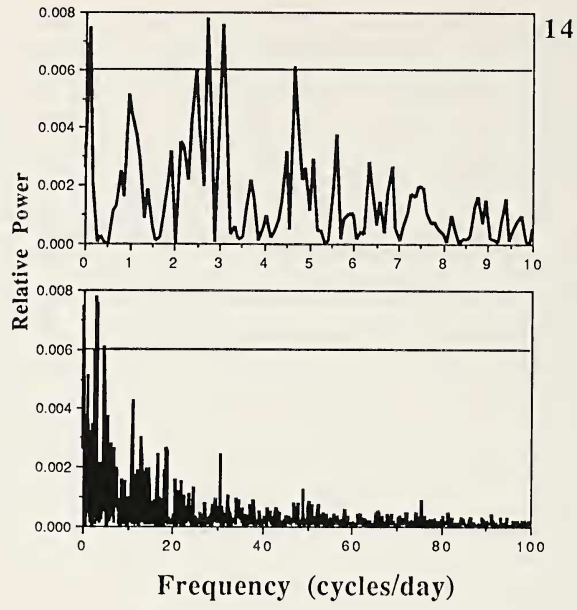
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Figures 9–15.—Activity of *F. pyramitela* under conditions of a 24-h photoperiod (LD) in which lights were on between 1100 and 1900 h (arrows). See the legend for Figs. 2–8 for an explanation of the layout.

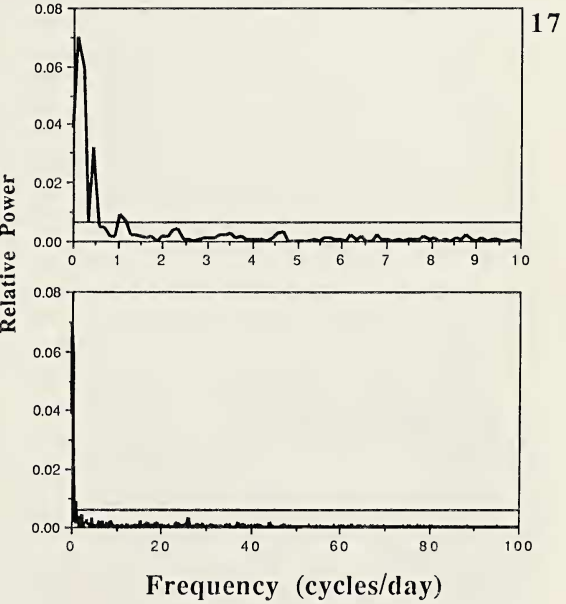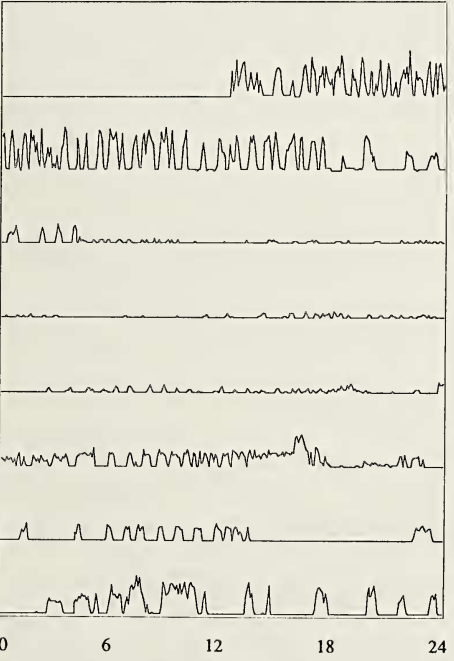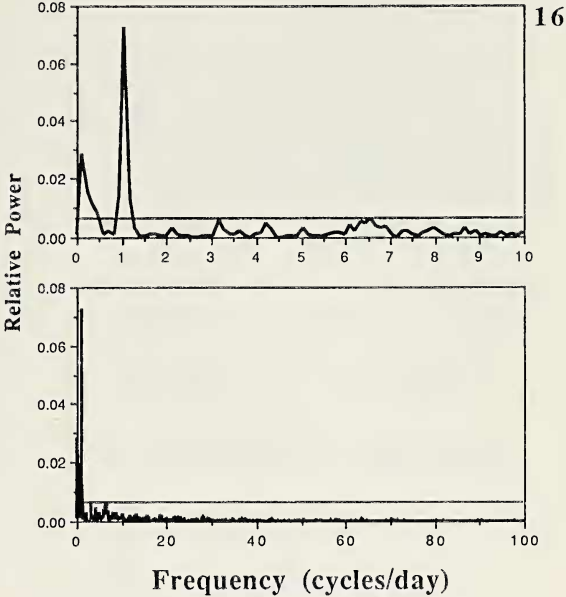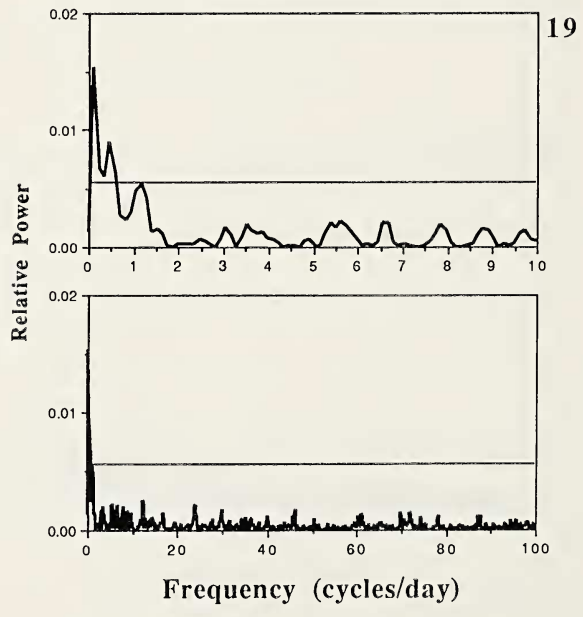Figures 9–15.—Continued.

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Figures 16–20.—Activity of *A. trigonum* under conditions of constant darkness (DD). See the legend for Figs. 2–8 for an explanation of the layout.
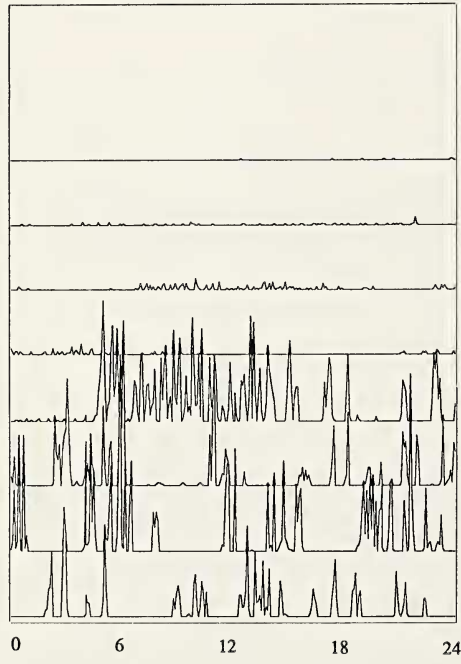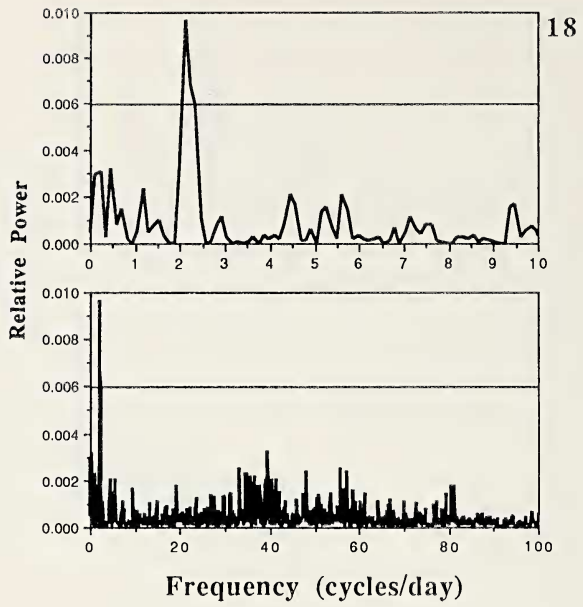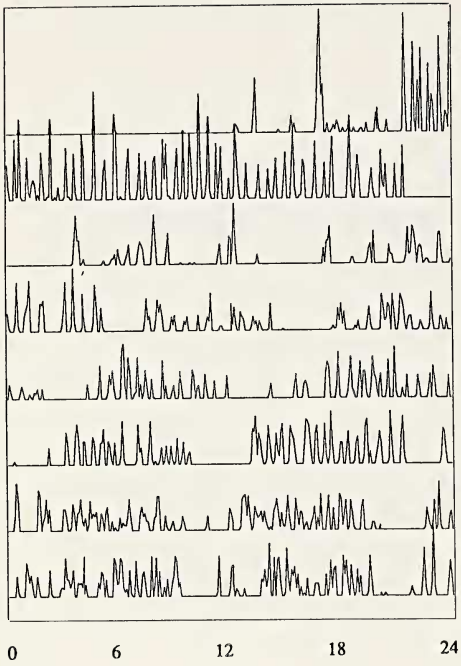
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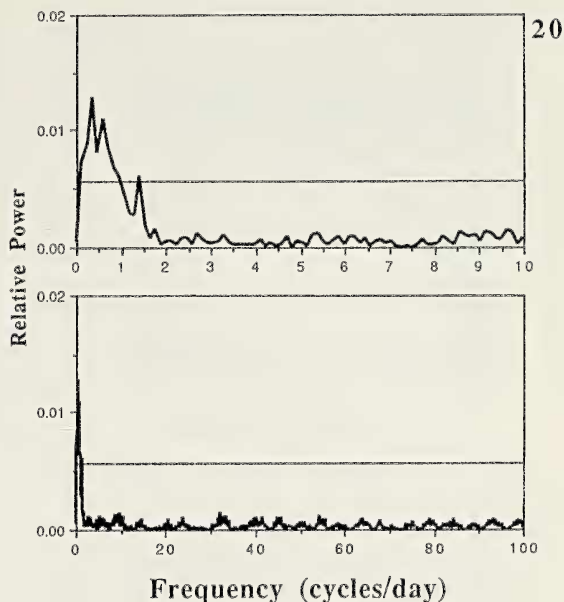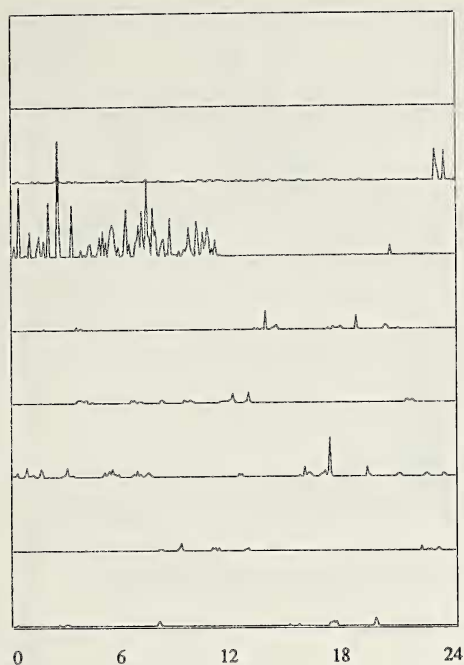
Figures 16–20.—Continued.

of slow rhythms (fewer than 24 cycles per day) activity in each 3-min period was summed and the string of 3-min sums was analyzed by FFT. For the detection of more rapidly cycling rhythms, subsets of the full data string were analyzed without summing (i.e., at 0.625 Hz, one sample every 1.6 s). The two procedures were required because the FFT algorithm could accommodate no more than 4096 data points in a single analysis. (2) Spectral Analysis II: the inactivity periods alone but in order, abstracted from the time-based string above, were analyzed in the same way by FFT and displayed as a power spectrum as described above; this procedure could reveal periodicities in a program which controlled the durations of inactivity periods. For each of the spectral analysis protocols, α was set at 0.01 to decrease the probability of type II errors.

RESULTS

The activity of both species of spiders in the isolation chambers consisted of bouts of nearly continuous activity alternating with periods during which no activity was detectable. Complete activity records for all tested spiders are shown in Figs. 2–20. Presented with each of the activity records in Figs. 2–20 are graphs of explained variance as a function of frequency [power spectra, from Spectral Analysis (I)]. In all but one of the

graphs that represent spider activity in constant darkness (*F. pyramitela*, Figs. 2–8; *A. trigonum*, Figs. 16–20), significant ($P < 0.01$) peaks occur near 1/day ($1.15 \times 10–5$ Hz), an indication that both species should be added to the long list of taxa in which the presence of endogenous circadian rhythms is confirmed. Some of the *F. pyramitela* under LD conditions (Figs. 9–15) were relatively inactive during the light phase of the cycle (Figs. 9–11), but others appeared to be uninfluenced by the dim lighting. The suppression of activity levels in some spiders by relatively dim light suggests that the animals are functionally nocturnal in the field.

Figures 2–20 also show that higher frequency periodicities participate in generating the observed activity patterns. In all of the power spectra, power is concentrated in significant peaks at frequencies between 1 cycle/day and 100 cycles/day: in Fig. 21, which shows cumulative power as a function of frequency for two *F. pyramitela* activity records, 50% of total power is at frequencies below 40 cycles/day and at least 75% is at frequencies below 100 cycles/day. Some of the significant high frequency (i.e., higher frequency than 1/day) periodicities are prominent enough to be easily seen in the activity records themselves. Perhaps the most prominent occurs in Fig. 18 in which a periodicity at about 40
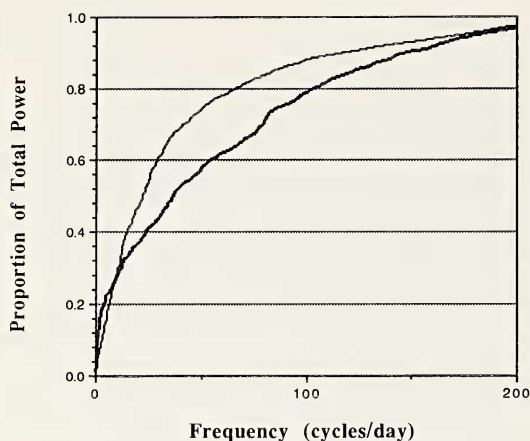
Figure 21.—Cumulative explained variance in the spectral analyses of the activity records of two *F. pyramitela*. The heavy line corresponds to the analysis of the data shown in Fig. 2 and the light line corresponds to the analysis of the data shown in Fig. 7. The most rapid rise in explained variance occurs very near the origin, indicating that high frequencies (> 75 cycles/day) are of minor importance (see text).

cycles/day is evident during the 24 h beginning at 2100 h on day 1, and during day 6. That 24-h period and all of day 6 for the same *A. trigonum* are shown with the corresponding power spectra in Fig. 22.

The intervals between activity bouts for all spiders in DD are shown and analyzed in Figs. 23–34. Spectral Analysis (II) of these intervals alone, kept in order but abstracted from a time-based activity series indicates that in *F. pyramitela* there is no indication of significant periodicity in the patterning of inter-activity intervals: the variance explained by periodicities is distributed relatively evenly across each spectrum of harmonics and is below the 0.01 level of significance for any particular peak above the first harmonic.

DISCUSSION

The behavior of an organism at a particular instant is a function of the interaction between its internal state and information the organism possesses about its surroundings. These two elements are not entirely distinct from each other, however: first, the internal state is in part an evolutionary construct, a consequence of historical responses to the organism's surroundings; and second, the organism's current information about its surroundings is available to it only after filtration through systems of sensation and per-
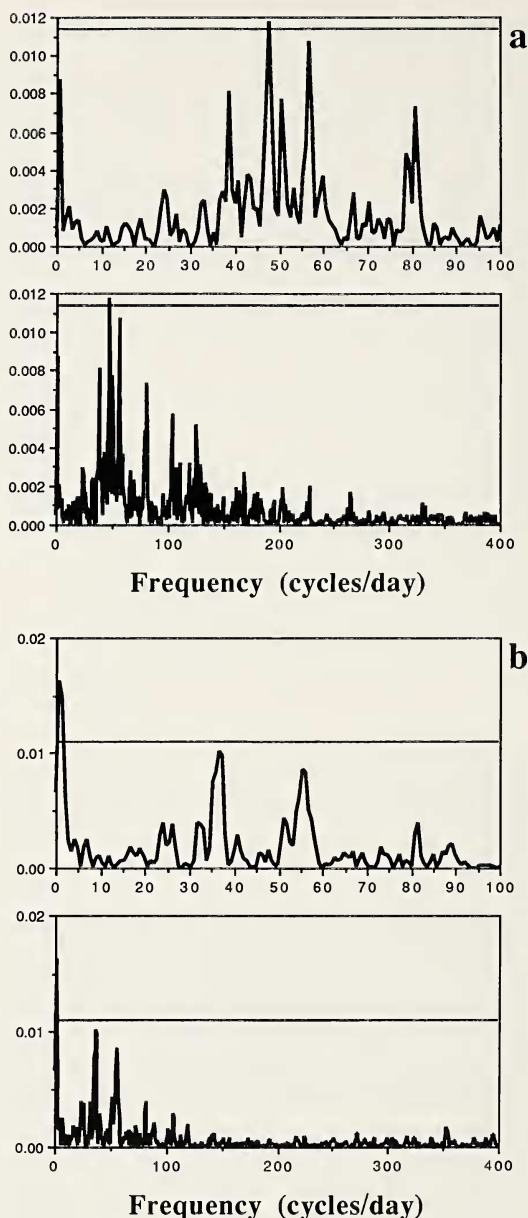

Figure 22.—Power spectra of subsets of the activity data shown in Fig. 18. In that data set, a periodicity at about 40 cycles/day is evident during the 24 h beginning at 2100 h on day 1, and during day 6. The corresponding power spectra are shown here in panels a and b, respectively. As in other power spectra in this study, peaks above the horizontal lines are significant at $\alpha = 0.01$. In panel b, the peak at 36 cycles/day is significant at $\alpha = 0.05$.

ception that are sensitive to (and part of) the internal state. In this context, the endogenous rhythms and other behavioral programs of an organism should be seen as parts of the internal
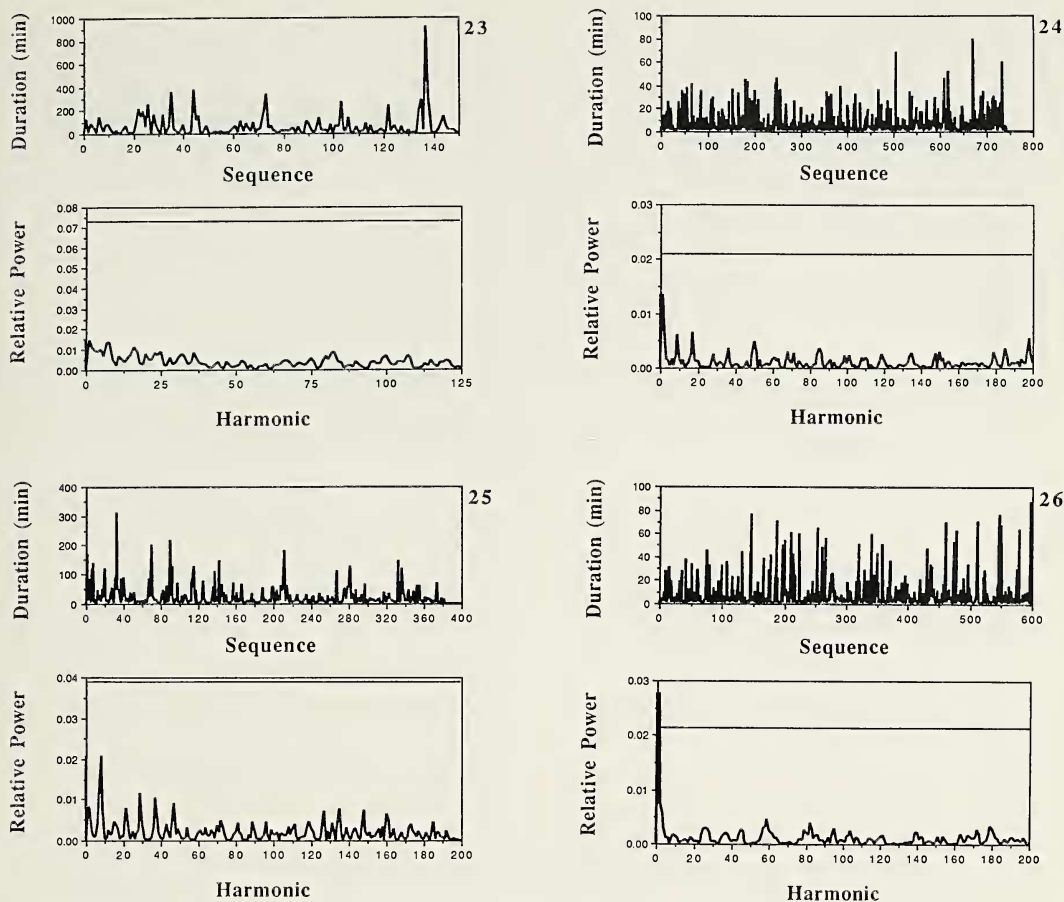
Figures 23–29.—Patterns in the durations of intervals between bouts of activity by *F. pyramitela* in constant darkness (DD). The durations of the intervals themselves, in each figure, are shown (in min) in the top panel, and the power spectrum for that sequence of intervals is shown in the bottom panel. Peaks above the horizontal lines are significant at $\alpha = 0.01$. In none of the analyses was there significant power at harmonics above DC, an indication that the sequences of durations were not different from random.

state that are evolutionary responses to the periodic or probabilistic structure of the environment.

Endogenous rhythms.—The best known endogenous rhythms in nature are those that approximate geophysical rhythms with respect to period length. Their original function was probably to provide a means by which organisms remain synchronized with their environments, and they now function, in addition, in photoperiod measurement and in navigation. Circadian rhythms and other low frequency endogenous rhythms (e.g., circumlunar, circannual) are nearly ubiquitous organizers of activity in organisms. Among spiders, circadian rhythms have been identified (reviewed in Cloudsley-Thompson 1987) but neither higher nor lower frequency

rhythms have been implicated in spider behavior. Because most spider species are short-lived, one would not expect to discover among them endogenous rhythms with very long periods (e.g., the lunar month or the solar year). Nor would one necessarily expect to discover endogenous rhythms with very short periods because the environment does not contain biologically important geophysical cycles shorter than the 12.25 h tidal period.

Because of the relatively brief durations of the studies described herein, I could not have demonstrated rhythms with periods longer than about two days. The data do make clear, however, that circadian and higher frequency rhythms in *F. pyramitela* and *A. trigonum* participate in organizing spontaneous motor activity (Figs. 2–20).
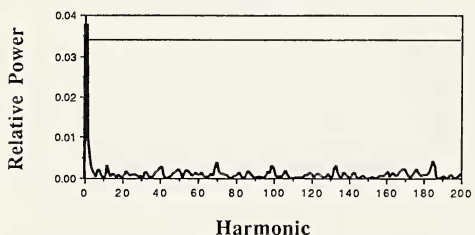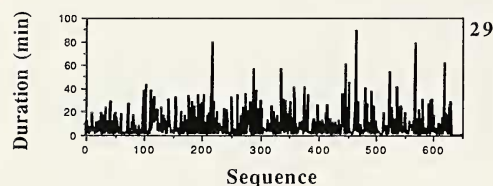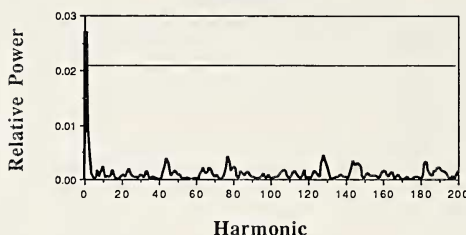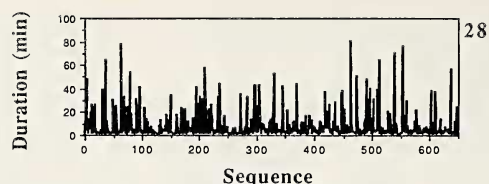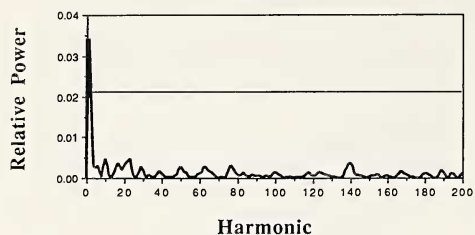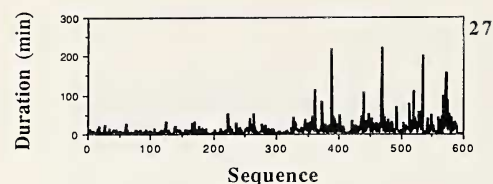
Figures 23–29.—Continued.

The evidence for an endogenous circadian clock is neither surprising nor particularly interesting given the ubiquity of these clocks in biological systems and their obvious efficacy in maintaining synchrony between the organism and its environment. The presence, and in some instances the predominance, of higher frequency rhythms, in contrast, is quite interesting.

Because of the absence of biologically important geophysical cycles with frequencies from 3–100 cycles/day, the significant periodicities in spider motor activity uncovered in that range cannot be of use to the spiders as environmental synchronizers. Moreover, because the spiders in these studies were given neither food nor water, the most likely physiological rhythms (those associated with the filling and emptying of the nutrient and water pools) must be discounted. I am left without a strong hypothesis with which to explain the function(s) of the high frequency periodicities demonstrated in the spontaneous motor activity of these isolated spiders. Of particular interest are the relatively stable oscillations that persist for many cycles and appear to be stable after several days (e.g., Figs. 18, 22): these are likely to be both important in the lives of the spiders and amenable to experimental investigation.

Other behavioral programs.—The periodicities described above are time-based; that is, they are detected as peaks of activity which are repeated at regular intervals in the time domain. Quite a distinct type of behavioral program would be one that obeyed the following algorithm: when behavior x begins, continue x until t seconds have elapsed, then begin behavior y ; assign t a new value based on some specified trigonometric function; repeat. The resulting periodicity in the durations of x would be undetectable if there were uncorrelated variability in the durations of
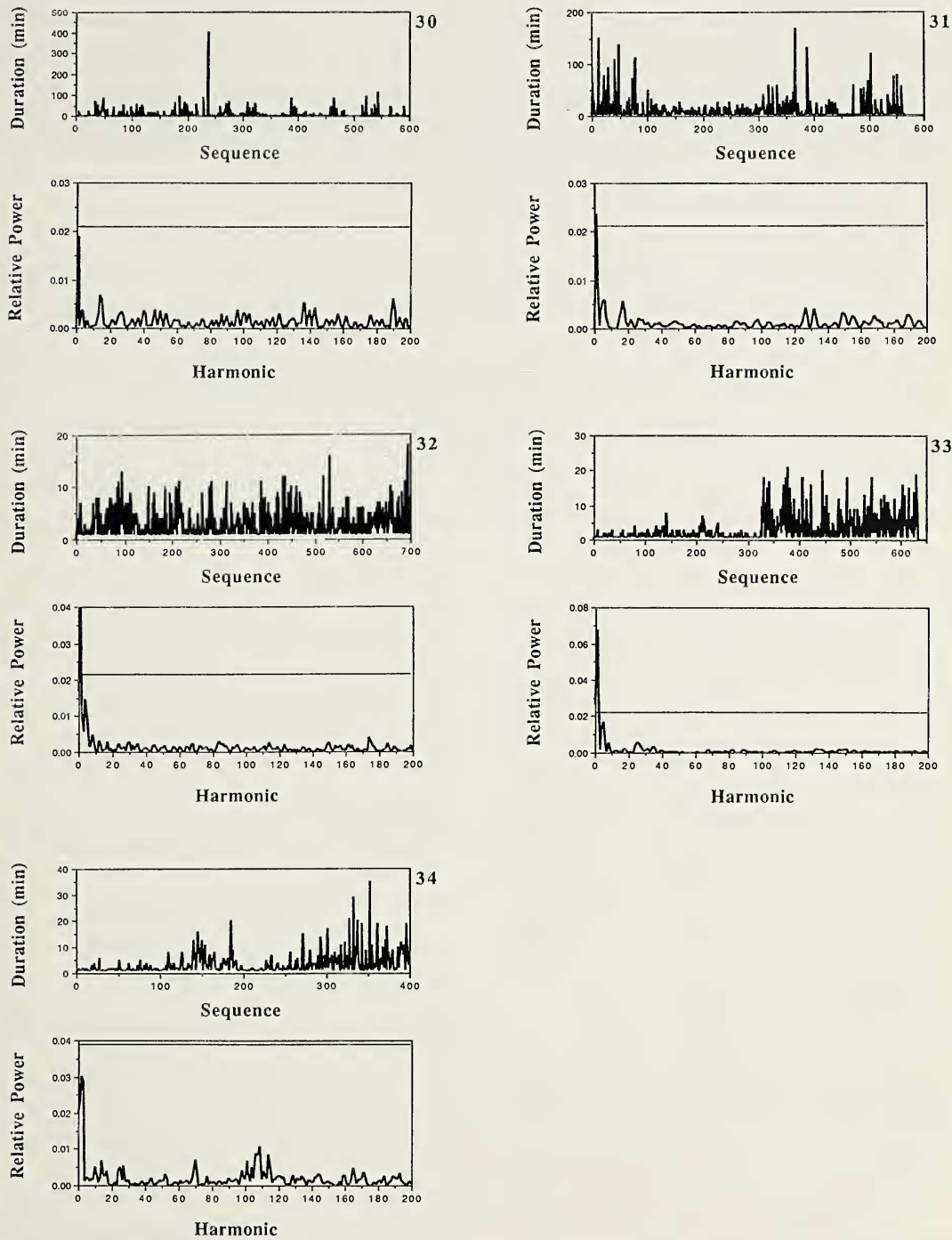
Figures 30–34.—Patterns in the durations of intervals between bouts of activity by *A. trigonum* in constant darkness (DD). See the legend to Figs. 23–29 for details of the layout. In none of the analyses was there significant power at harmonics above DC, an indication that the sequences of durations were not different from random.

y. For example: suppose *y* is motor activity and *x* is inactivity; if activity occurs in bouts that vary in duration according to some non-periodic function, then peaks in the durations of inactivity bouts would appear nonperiodic; if, in contrast, inactivity bouts were extracted in order from the time series, the periodicity of peaks in the durations of inactivity bouts would become apparent. This is the reasoning behind Spectral Analysis II. In the linyphiid spider, *F. pyramitela*, and in the theridiid spider, *A. trigonum*, the durations of inactivity bouts show no evidence of periodicity (Figs. 23–34) which leads to the conclusion that these bouts are not generated by the sort of behavioral program outlined above.

Thus there is no evidence from the data presented here that the inactivity bouts of *F. pyramitela* and *A. trigonum* are regulated by any sort of non-random program. What, then, determines the duration of a particular period of inactivity in these spiders? The possibility that they are coupled to, or driven by, some process the output of which is itself random is intriguing but well outside the scope of this study.

Conclusion.—My analysis of the motor activity patterns of *F. pyramitela* and *A. trigonum* reveals that endogenous oscillators participate in determining the timing of activity but not the durations of inter-activity intervals. Some nonperiodic processes, which may be the equivalent of random-number generators, are also important in determining the durations of inter-activity intervals. Both the nonperiodic processes and the endogenous oscillators with periods much shorter than 24 h are particularly interesting because they constitute an unexplored set of behavioral programs that may influence much of the behavior of these spiders.

ACKNOWLEDGMENTS

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